

Preliminary Crystallographic Analysis of the Breakage–Reunion Domain of the *Escherichia coli* DNA Gyrase A Protein

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The 64×10^3 M_r N-terminal breakage–reunion domain of the *Escherichia coli* DNA gyrase A protein was purified from an over-expressing strain. When complexed with the gyrase B protein, this truncated A protein has all of the enzymic properties of the full-length counterpart, although with reduced efficiency in some cases. The 64×10^3 M_r protein has been crystallized in several forms, a number of which were too small for crystallographic analysis. However, two forms grew to sufficient size for preliminary X-ray analysis. Both forms were tetragonal with a primitive lattice. One form (type I) had cell dimensions of $a = b = 170$ Å, $c = 145$ Å a space group of either $P4_12_12$ ($P4_32_12$) or $P4_22_12$, and diffracted to 6 Å resolution. The type II crystals had cell dimensions of $a = b = 177$ Å, $c = 175$ Å, a space group of $P4_12_12$ ($P4_32_12$) or $P4_22_12$, and diffracted to at least 4.5 Å resolution. Both crystal forms apparently contained four subunits (possibly a tetramer) in the asymmetric unit. We are attempting to increase the size and quality of these crystals.

DNA gyrase is a type II topoisomerase that catalyses the negative supercoiling of closed-circular DNA using the free energy derived from ATP hydrolysis (for reviews, see Gellert, 1981; Wang, 1985). The enzyme from *Escherichia coli* consists of two proteins, A and B, with relative molecular masses of 97×10^3 and 90×10^3 , respectively; the active enzyme being an A_2B_2 complex (Klevan & Wang, 1980; Kreuger *et al.*, 1990). The *gyrA* and *gyrB* genes have been sequenced and cloned in such a way that the A and B proteins can be over-produced (Mizuuchi *et al.*, 1984; Yamagishi *et al.*, 1986; Swanberg & Wang, 1987; Adachi *et al.*, 1987; Hallett *et al.*, 1990). The gyrase A protein is principally involved in the breakage and reunion of DNA while the B protein is the site of ATP hydrolysis (Gellert, 1981). The mechanism of supercoiling by gyrase is thought to involve the ATP-driven passage of a segment of DNA through a double-stranded DNA break stabilized in part *via* covalent protein–DNA bonds (for a review, see Maxwell &

Gellert, 1986). DNA supercoiling by gyrase can be inhibited by two groups of antibiotics, the quinolones and the coumarins, which appear to act on the A and B proteins, respectively. The role of gyrase A protein in DNA breakage and reunion has been substantiated by the observation that incubation of gyrase and DNA in the presence of quinolone drugs, followed by termination of the reaction with SDS, leads to double-stranded cleavage of the DNA (Gellert *et al.*, 1977). Moreover, the A subunits are found to be covalently attached to the DNA *via* O^4 -phosphotyrosine linkages between Tyr122 of the A protein and the newly formed 5'-phosphates (Horowitz & Wang, 1987).

It has been shown recently that cleavage of the DNA gyrase A protein with trypsin yields two stable fragments with relative molecular masses of 64×10^3 and 33×10^3 (Reece & Maxwell, 1989). The 64×10^3 M_r fragment, derived from the N terminus of the protein, retains the catalytic activities of the A protein. In combination with the B protein, it will support DNA supercoiling (albeit with reduced efficiency) and quinolone-induced DNA cleavage. The C-terminal 33×10^3 M_r fragment appears to have no enzymic activity. It is proposed that the 64×10^3 M_r

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fragment represents the DNA breakage-reunion domain of the A protein while the $33 \times 10^3 M_r$ fragment is involved in maintaining the stability of the DNA-protein complex (Reece & Maxwell, 1989). Following insertion of a stop codon into the *gyrA* gene, the $64 \times 10^3 M_r$ protein (comprising amino acids 1 to 572 of the intact A protein; GyrA(1-572)) can be made as a gene product in large amounts (Reece & Maxwell, unpublished results). The $64 \times 10^3 M_r$ domain of the gyrase A protein contains the amino acid residues involved in DNA breakage-reunion and in the interaction with quinolone drugs (Yoshida *et al.*, 1988). Consequently, structural information relating to this protein will be important to our understanding of the mechanism of gyrase (and other topoisomerases) as well as the mode of action of the quinolone antibiotics.

The $64 \times 10^3 M_r$ N-terminal domain of the DNA gyrase A protein was purified from *E. coli* cells harbouring the plasmid pRJR242 (Reece & Maxwell, unpublished results), based on the method of Hallett *et al.* (1990). Cells were grown at 37°C in 2 × YT broth (10 g Bacto-tryptone (Difco)/l, 10 g Bacto-yeast extract (Difco)/l and 5 g NaCl/l) containing 100 µg ampicillin/ml and induced by the addition of isopropyl-β-D-thiogalactopyranoside (to 50 µM). After harvesting, and cell disruption using a French press, protein was precipitated by the addition of ammonium sulphate to a final concentration of 50% (w/v). The protein was purified by chromatography using heparin-Sepharose (Pharmacia), FPLC Mono Q and FPLC Phenyl Superose (Pharmacia). The final stage of the purification process involved the use of two FPLC Superose 12 (10/30) columns run in series. The columns were equilibrated in 50 mM-Tris·HCl (pH 7.5), 100 mM-NaCl, 0.2 mM-EDTA, 5 mM-dithiothreitol. Protein (no more than 5 mg per run) was applied to the columns in a small volume (0.5 ml). The protein was estimated to be

>99% pure as judged by scanning densitometry of SDS/polyacrylamide gels. The yield from such preparations was approximately 20 mg of purified protein per litre of cells. For crystallization trials, the protein was concentrated to approximately 10 mg/ml by pressure dialysis and the buffer exchanged for 20 mM-Tris·HCl (pH 7.5).

Crystals were obtained by vapour diffusion using hanging drops, with ammonium sulphate as precipitant. Several crystal forms were obtained at room temperature over a range of pH values between 7.5 and 9.0. However, only two of these forms grew to sufficient size for X-ray analysis. Both were grown at pH 7.5 at an ammonium sulphate concentration of 28 to 30% saturation at room temperature. The first form (type I) grew to a size of 0.05 mm × 0.05 mm × 0.2 mm (Fig. 1(a)). These crystals diffracted weakly to 6 Å resolution using Synchrotron radiation (EMBL, Hamburg) and were tetragonal with a primitive lattice. The cell dimensions were $a = b = 170$ Å, $c = 145$ Å (1 Å = 0.1 nm). Because of the weak diffraction, the space group could only be tentatively assigned as either $P4_12_12$ ($P4_32_12$) or $P4_22_12$. Any of these space groups would result in a $V_m = 2.05$ Å³ per dalton assuming one tetramer in the asymmetric unit (Matthews, 1968).

The second crystal form to be analysed (type II) grew as rather squat, square-based bipyramids with dimensions roughly equal to each other. The largest crystals grew to a size of 0.2 mm in each of these dimensions (Fig. 1(b)). Probably because of their larger size, these crystals diffracted more strongly, though still to only 4.5 Å resolution (Fig. 2). This crystal form was again tetragonal with a primitive lattice, but with cell dimensions $a = b = 177$ Å, while $c = 175$ Å. The space group again appeared to be $P4_12_12$ ($P4_32_12$) or $P4_22_12$. Despite the larger cell size, the V_m value (2.68 Å³ per dalton) is still within the usual range for proteins (Matthews,

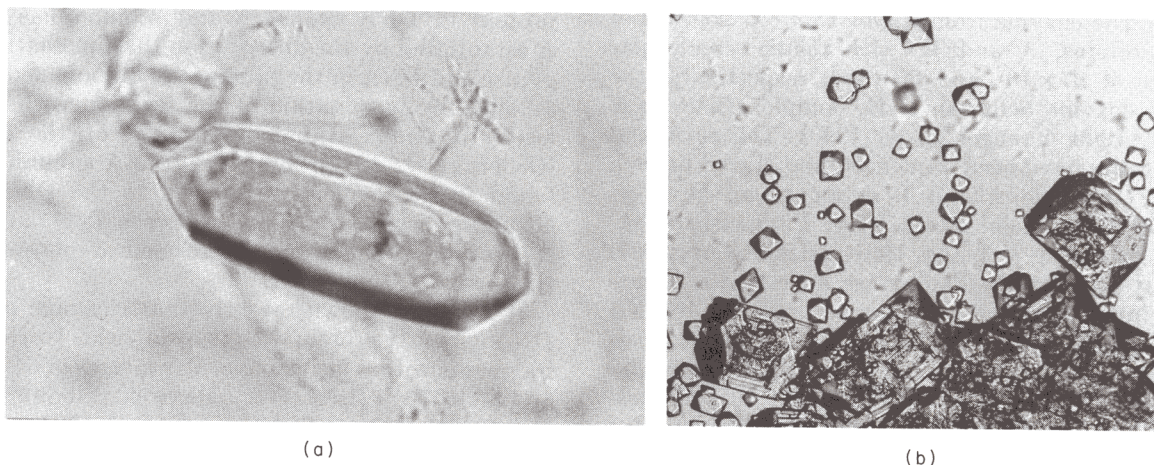


Figure 1. Crystals of the breakage-reunion domain of the DNA gyrase A protein. (a) Type I crystals grown from a solution containing 9 mg protein/ml, 20 mM-Tris·HCl (pH 7.5), 28% ammonium sulphate. The approximate size of this crystal was 0.05 mm × 0.05 mm × 0.2 mm. (b) Type II crystals grown from a solution containing 9 mg protein/ml, 20 mM-Tris·HCl (pH 7.5), 30% ammonium sulphate. A number of these crystals grew to a size of 0.2 mm in each direction.

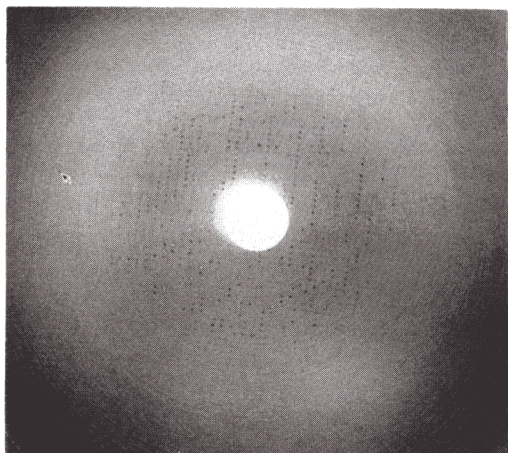


Figure 2. A 1° oscillation image of the type II crystals shown in Fig. 1(b) using X-rays of 0.96 Å wavelength (Synchrotron radiation from beamline X-11, EMBL, Hamburg) recorded on an imaging plate scanner. The diffraction pattern extends to approximately 4.5 Å resolution.

1968), if the asymmetric unit were to contain one tetramer. Efforts are being aimed at improving the size and quality of these crystals.

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